A Deep Dive Into the Science Behind Arthrex Calcium Sulfate BioBeads

Study Summary

Introduction

Arthrex Calcium Sulfate BioBeads are one of the only synthetic bone void fillers on the market indicated for use directly at the site of infection. Advanced manufacturing processes are applied to obtain the purest calcium sulfate molecules for this product. The studies below show that Arthrex Calcium Sulfate BioBeads are ideal for its indicated applications due to the characteristics of this product as outlined in this white paper. This paper also compares the Arthrex Calcium Sulfate Biobeads to another widely used calcium sulfate product, Stimulan.

The testing, as summarized below, shows that Arthrex Calcium Sulfate BioBeads were biocompatible and passed all the acceptance criteria for the outlined tests.

Biocompatibility Testing

Cytotoxicity MEM Elution Test
The MEM Elution test is designed to determine the cytotoxicity of extractable substances. An extract of Arthrex Calcium Sulfate BioBeads was added to cell monolayers and incubated. The cell monolayers were examined and neither cell lysis nor any intracytoplasmic granules were found. The device is considered to be noncytotoxic.

Bacterial Mutagenicity (Ames) Test
The *Salmonella Typhimurium* Reverse Mutation (Ames) test employs several strains of *S. enterica* serovar Typhimurium, which require the amino acid histidine for growth to detect point mutations. The Arthrex Calcium Sulfate BioBeads extract tested against the five strains did not meet the criteria for a potential mutagen. The device is found to be nonmutagenic.

ISO Maximization Test for Delayed Hypersensitivity
ISO 10993-10:2010 was used to determine if Arthrex Calcium Sulfate BioBeads would cause delayed dermal contact sensitization in a guinea pig maximization test. The study results showed that Arthrex Calcium Sulfate BioBeads extracts showed no evidence of causing delayed dermal contact sensitization in the guinea pig. The device is considered to be nonsensitizing.

ISO Acute Systemic Toxicity Test
The purpose of the study was to determine whether leachables extracted from Arthrex Calcium Sulfate BioBeads would cause acute systemic toxicity following single-dose systemic injection into mice. The study result showed that there was no mortality or evidence of systemic toxicity from the extracts. Each test article extract met the test requirements. The device is considered to be systemically nontoxic.

ISO Intracutaneous (Intradermal) Reactivity Test
The purpose of the study was to determine whether leachables extracted from Arthrex Calcium Sulfate BioBeads would cause local dermal irritant effects following injection into rabbit skin. The study result showed that there was no evidence of significant irritation from the extracts injected intracutaneously into rabbits. The device is not considered to be an irritant.

Chromosome Aberration Analysis
This test determines if the device causes structural chromosome aberrations in Chinese hamster ovary (CHO) cells. The test complies with OECD and ISO guidelines as an in vitro diagnostic for genotoxicity. Results indicate no aberration in chromosome structure following exposure to the device.

ISO Materials-Mediated Rabbit Pyrogen Study
This test determines whether a saline extract of the device causes a pyrogenic response (fever) in rabbits. This test is in compliance with ISO 10993-11. All extracts tested negative. The material is nonpyrogenic.

ISO In Vivo Mouse Micronucleus Assay
This test determined if the device induces micronuclei formation in immature polychromatic erythrocytes present in the bone marrow of adult mice. The presence of polychromatic erythrocytes is an indication of a mutagenic substance leached from the device. The test complies with ISO 10993-3. All extracts tested negative, indicating the device is nonmutagenic.

Arthrex Calcium Sulfate BioBeads bone graft material passed the requirements of all the above biocompatibility tests. It can be concluded that the product is biocompatible and nontoxic.

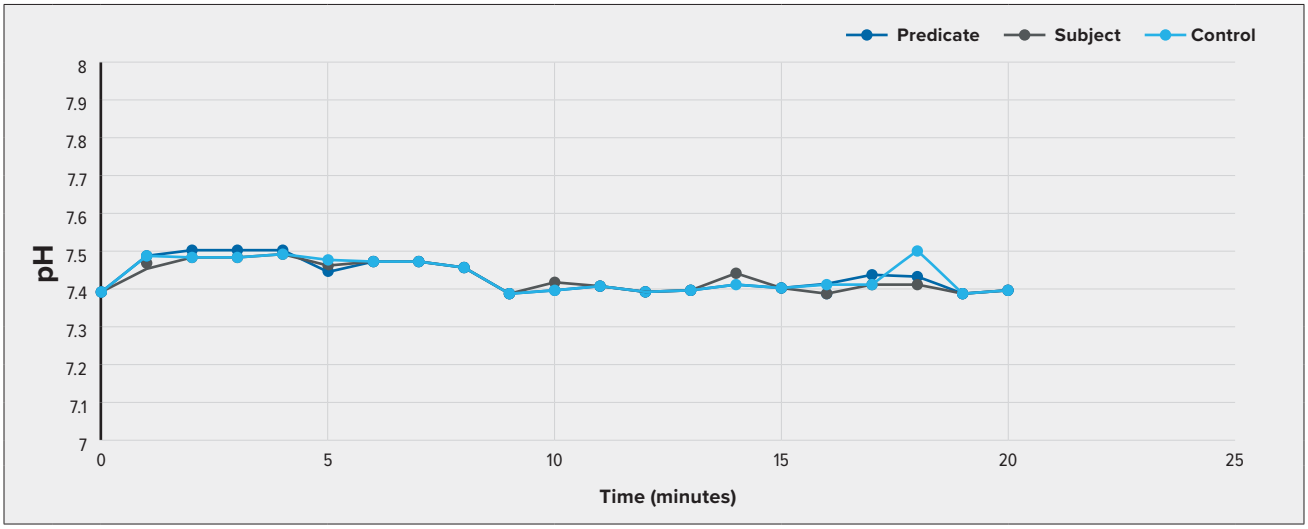
Benchtop Performance Testing

The manufacturer completed performance tests that simulated the intended physiological environment as outlined in the Food and Drug Administration's Class II Special Controls Guidance Document: Resorbable Calcium Salt Bone Void Filler Device (2003). This section summarizes performance test results for Arthrex Calcium Sulfate BioBeads and the predicate devices. The critical specifications of Arthrex Calcium Sulfate BioBeads were compared to the predicate device. These analyses consisted of chemistry, crystallinity, physical form, porosity, dissolution/solubility, pH, working time, setting time, dimensional stability, and setting reaction temperature.

pH Testing

This test compared pH changes in surrounding simulated body fluids (phosphate-buffered saline [PBS]) of the predicate and subject test devices while the devices cured in vitro (Figure 1). The pH of the surrounding PBS for both devices was within the physiological value of ~7.5.

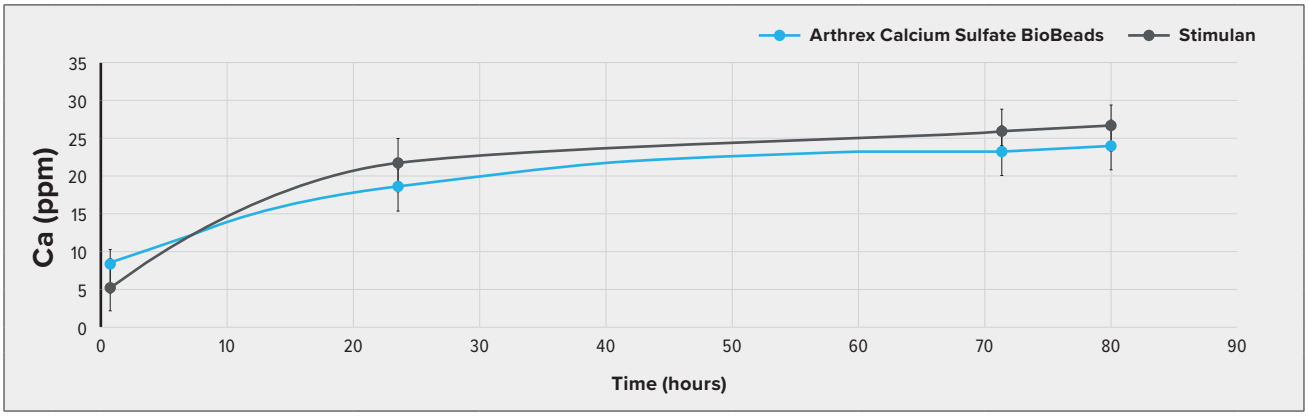
Figure 1. pH change versus time for PBS with subject (Arthrex Calcium Sulfate BioBeads), predicate (Stimulan), and control (no cement)



Dissolution/Solubility Testing

Using inductively coupled plasma mass spectrometry (ICP-MS), solubility of predicate and subject Arthrex Calcium Sulfate BioBeads test devices was analyzed over 80 hours while immersed in Dulbecco's PBS and maintained at physiological conditions (pH = 7.4, 37 °C) (Figure 2). The test purpose was to evaluate the in vitro dissolution behavior of the Arthrex Calcium Sulfate BioBeads device compared to the predicate device. The calcium ion concentration at 80 hours for the test device was 23.8 ppm and for the predicate device was 26 ppm. The tested devices' differences for the in vitro solubility of the two minerals were negligible and within experimental error (within 95% confidence interval). Over the course of the study, both products exhibited sparingly soluble calcium release. The x-ray diffraction (XRD) results presented in Figure 2 display the diffraction pattern for Arthrex Calcium Sulfate BioBeads.

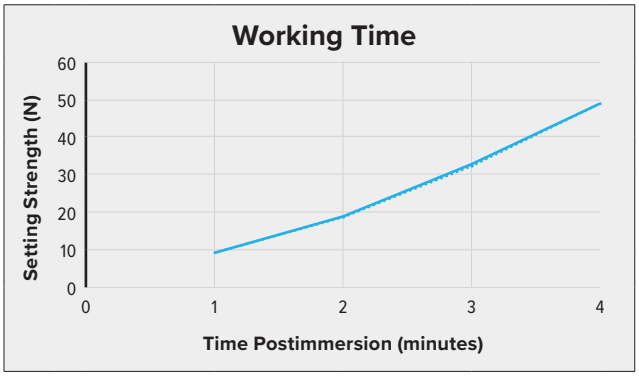
Figure 2. In vitro solubility testing of Arthrex Calcium Sulfate BioBeads and predicate (Stimulan)



Working Time

The test purpose was to examine the intraoperative handling properties of Arthrex Calcium Sulfate BioBeads to ensure that the material has sufficient working time during surgical implantation in bone defects and/or voids (Figure 3).

Figure 3. Working time of Arthrex Calcium Sulfate BioBeads immersed in 32 °C PBS solution

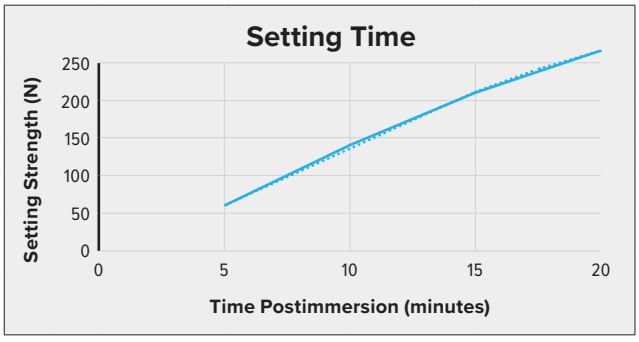


The working time of Arthrex Calcium Sulfate BioBeads at 32 °C was determined per the modification of the setting test (ASTM C403/C403M-99). The data showed an initiation set time of 3 minutes to attain a recordable load greater than 25 N when the curing temperature was 32 °C. This indicated a maximum allowable working time before the material begins to harden in vivo of 3 minutes postimplantation. This provides ample opportunity for surgical adjustment, if needed.

Setting Time

The test purpose was to examine the setting properties of Arthrex Calcium Sulfate BioBeads to ensure the bone void filler would set sufficiently hard in vitro and within a clinically relevant time under physiologic pH and temperature conditions (Figure 4). This test was a modification of the standard setting test described in ASTM C403/C403M-99, in which the load required to drive needles at a prescribed distance into concrete or a similar setting material was measured.

Figure 4. Setting time of Arthrex Calcium Sulfate BioBeads immersed in 32 °C PBS solution immediately after mixing



Immersed for 10 minutes in physiological conditions of temperature and pH, Arthrex Calcium Sulfate BioBeads reached adequate strength, as presented in Appendix J. Data analysis yielded a set time of ~10 minutes to reach a load greater than 135 N.

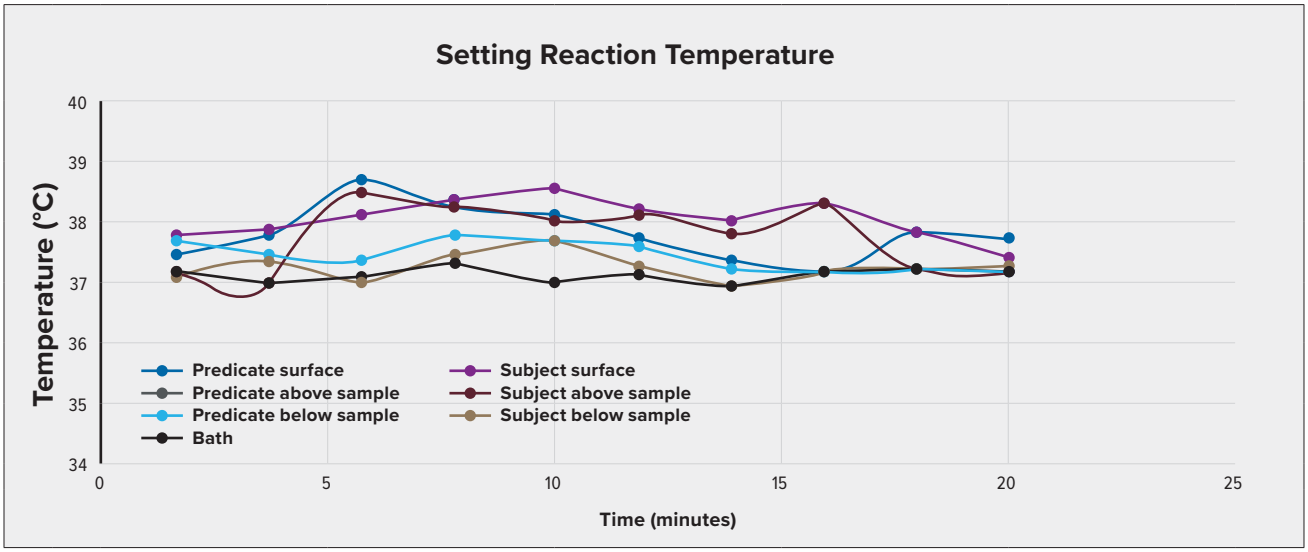
Dimensional Stability

Dimensional stability testing measured the volume change following incubation and setting at physiologic pH and temperature. Arthrex Calcium Sulfate BioBeads hardened at 30 minutes in a contained volume with no physical shape change at 24 hours. Therefore, no change in physical shape would be expected following implantation in vivo.

Setting Reaction Temperature

Some orthopedic cement devices undergo an exothermic setting reaction that is of interest due to its biologic consequence. Both Arthrex Calcium Sulfate BioBeads and the predicate devices underwent hydration and setting via an isothermic reaction. The data indicated that neither device's setting reaction significantly changed fluid temperature within the setting material's immediate vicinity (Figure 5). Temperature fluctuation was minimized and expected to ensure tissue compatibility. The results demonstrated that Arthrex Calcium Sulfate BioBeads remain within the physiologic temperature range and are expected to have no adverse biologic consequence.

Figure 5. Setting reaction temperature change for subject (Arthrex Calcium Sulfate BioBeads) and predicate (Stimulan) after immersion in PBS at 37 °C



Chemical Analysis

Chemical and microstructural analysis per the FTIR, XRD, SEM, and porosimetry supported a detailed description of the composition and microstructure of Arthrex Calcium Sulfate BioBeads and predicate devices. Additionally, the analysis helped to predict similarities in in vivo performance testing. All tests were performed on predicate and Arthrex Calcium Sulfate BioBeads devices to establish substantial equivalence.

Properties Analyzed	Technique and Tool
Chemistry	Fourier Transform Infrared Spectroscopy (FTIR) and X-ray Diffraction (XRD)
Crystallinity	X-ray Diffraction (XRD)
Physical Form and Microstructure	Scanning Electron Microscopy (SEM)
Porosity	Mercury Intrusion Porosimetry

XRD and FTIR analyses confirmed that both predicate and subject devices were composed of calcium sulfate dihydrate (CSD), with no other phases detected (Figures 6 and 7, respectively). SEM (Figure 8) and porosimetry data indicate both tested devices formed intermingling, interlocking, nanosized crystals of CSD after hydration and curing in vitro. Bulk density, pore diameter, and total porous volume measured by mercury intrusion porosimetry showed that both subject and predicate devices were nanoporous/microporous materials with similar bulk densities.

Figure 6. XRD pattern for final device: Arthrex Calcium Sulfate BioBeads after curing and drying

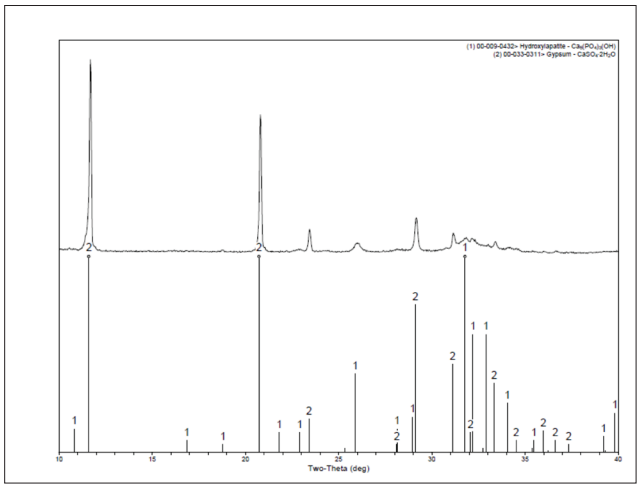


Figure 7. FTIR patterns for Arthrex Calcium Sulfate BioBeads after curing and drying, showing characteristic absorption bands for sulfate ion attributable to SO_4 in $CaSO_4$ $2H_2O$

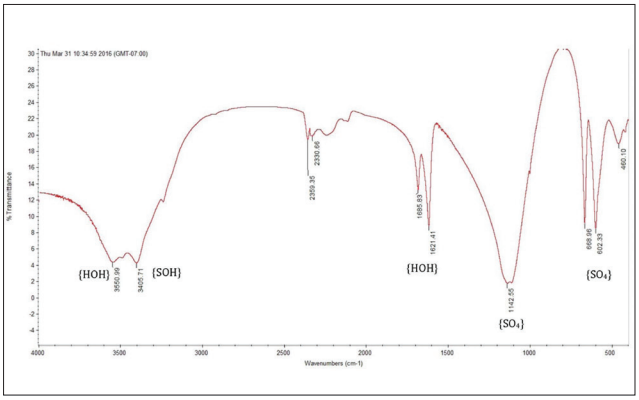
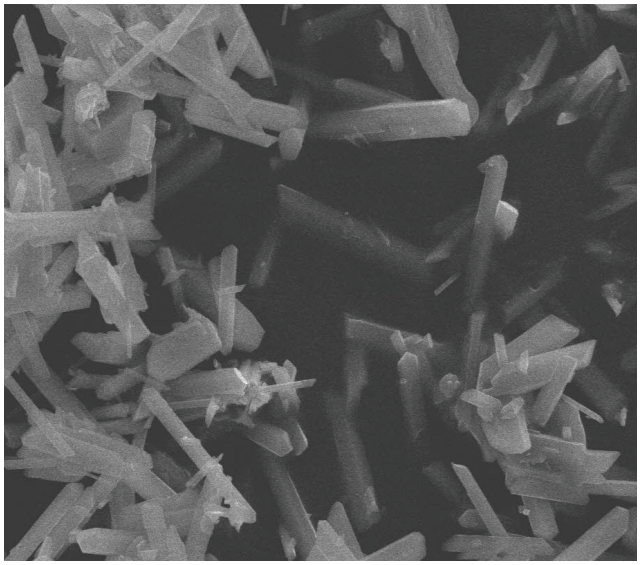
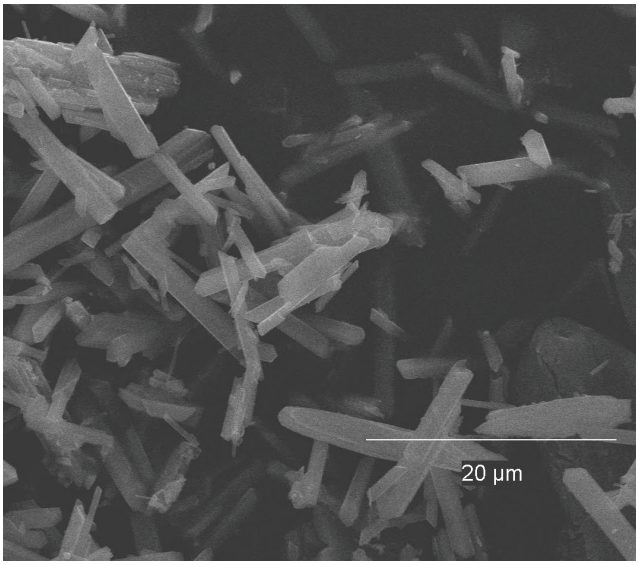


Figure 8. SEM micrograph of cured and dried subject A (Arthrex Calcium Sulfate BioBeads) and predicate B (Stimulan)



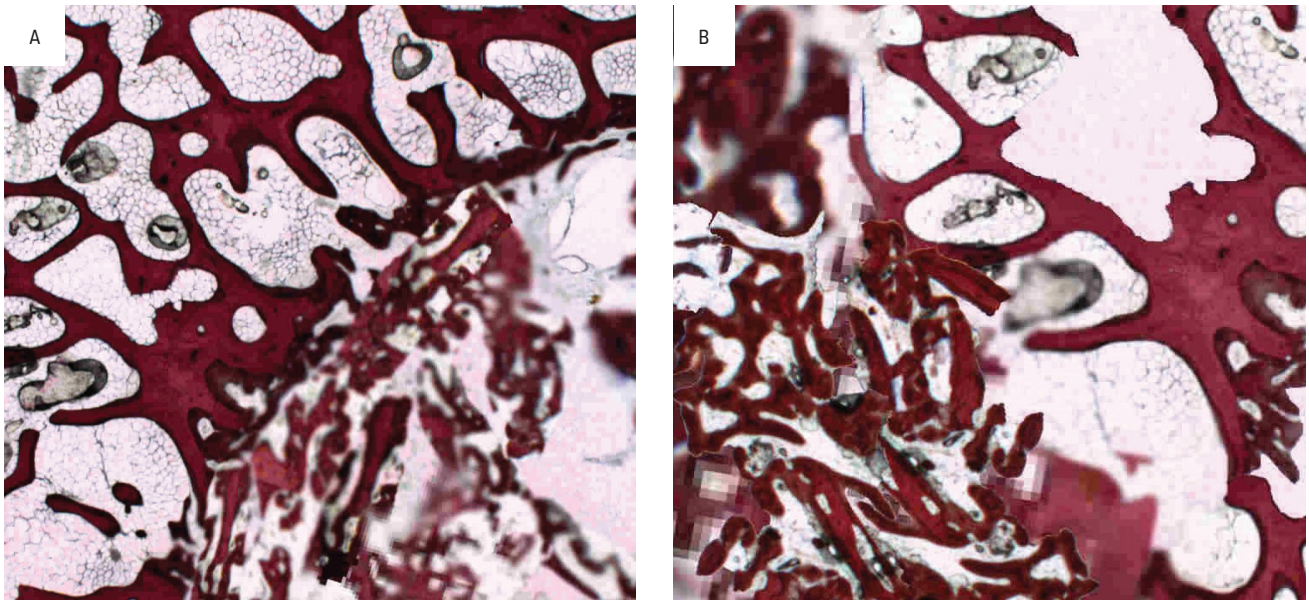


Animal Testing: Ovine Cancellous Bone Defect

As described in the Class II Special Controls Guidance Document: Resorbable Calcium Salt Bone Void Filler Device (2003), Pacific Bioceramics demonstrated that the subject device had the same critical specifications (ie, chemistry, crystallinity, physical form, porosity, dissolution/solubility) and the same intended use as the predicate device. A large animal critical-sized ovine defect model evaluated the biocompatibility, tissue reaction, implant resorption, bone formation, and surgical handling properties of Arthrex Calcium Sulfate BioBeads following implantation in the femoral and tibial metaphyses. This ovine model compared the subject device (Arthrex Calcium Sulfate BioBeads) with the predicate device (Stimulan). Both devices are comprised of a calcium salt cementitious phase that is combined at time of use with an aqueous solution.

After implantation into sheep cancellous bone sites, histological data exhibited no adverse tissue response from either device. Both materials were biocompatible with normal bone remodeling that occurred around the periphery of the cylindrical implant area. There was no visible inflammatory reaction associated with the implanted devices, and no macrophages or giant cells were observed within the implant. Tissue fibrosis was not observed within the implanted regions. Histomorphometric analysis showed complete device resorption and similar bony ingrowth rates between subject and predicate devices over the implantation period of 3 months. The Arthrex Calcium Sulfate BioBeads device maintained the reported safety profile of the predicate device with no remarkable safety issues.

Figure 9. Undecalcified histology of the subject A (Arthrex Calcium Sulfate BioBeads) and predicate B (Stimulan) at 3 months, showing that the majority of the defect filled with normal host tissue. No adverse tissue reaction was identified.



Discussion and Conclusion

Calcium sulfate bone void fillers come in many commercially available forms today, including putties, injectable pastes, beads, and blocks. These products are biocompatible, which means they do not cause the body to react in an adverse manner. They are also resorbed by the body relatively quickly. The amount of time calcium sulfate bone void fillers resorb is typically a matter of the size of material, location, and local physiological environment. The Arthrex Calcium Sulfate BioBeads Kits are provided sterile for single patient use. The kit contains calcium sulfate powder and mixing solution in premeasured quantities so when mixed together in a sterile mixing bowl, the resultant paste can be digitally packed into open bone void/gaps to set in situ. The mixture sets to form a solid calcium sulfate implantable medical device. The Arthrex Calcium Sulfate BioBeads are manufactured from medical-grade calcium sulfate dihydrate ($CaSO_4$ $2H_2O$) that resorbs and is replaced with bone during the healing process. The bone void filler material is biodegradable and biocompatible and can be used within an infected bone site.

Reference

1. Pacific Bioceramics. Data on file (Biocompatibility and performance testing). Santa Cruz, CA; 2022.

arthrex.com